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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Beutler, et al.

Serial No.: 09/396,985

Filed: September 15, 1999

For: LPS-RESPONSE GENE COMPOSITIONS AND METHODS

Group Art Unit: 1646

Examiner: Basi, N.

Atty. Dkt. No.: UTSD:602/TMB

DECLARATION OF DAVID D. CHAPLIN, M.D., Ph.D.

I, David D. Chaplin, hereby declare as follows:

1. I am a U.S. citizen residing at 406 Wildwood Lane, Indian Springs, Alabama 34124. I am the Charles H. McCauley Professor and Chairman of the Department of Microbiology at the University of Alabama at Birmingham. I have extensive experience in the study of cellular responses to endotoxins. References containing examples of my work are included in my *Curriculum Vitae*. A copy of my *Curriculum Vitae* is attached as Exhibit 1.

2. I understand that the present invention relates to methods for screening for modulators of TLR-4 mediated responses to lipopolysaccharide (LPS) mediated responses. The methods involve the use of a TLR-4 polypeptide and the measurement of LPS mediated responses, themselves mediated by TLR-4, in the presence and absence of a putative modulatory compound.

3. I understand that the patent examiner in charge of assessing the patentability of the above-referenced application has rejected the claims of that application on a variety of grounds. I have reviewed the Office Action dated April 23, 2002, the specification of the application and the pending claims. In light of these documents, and my knowledge of the field of endotoxins and cellular biology, I make the following statements.

4. I understand that the examiner has asserted that skilled cellular biologists would not clearly understand the scope of the claims since they recite measurement of a "lipopolysaccharide mediated response." The examiner has asserted that a "lipopolysaccharide mediated response" is not clearly defined in the specification or in the knowledge of the field of endotoxin biology. I do not find this to be the case.

5. The specification clearly sets forth the actors and elements of lipopolysaccahride mediated responses that are mediated by TLR-4. For example, see pages 87-88, which refer to TNF production and splenocyte proliferation assays, commonly employed assays for LPS response.

6. Furthermore, a skilled researcher in endotoxin biology, relying upon the generally available knowledge in the field, would understand that in the context of the application the "lipopolysaccharide pathway" is the cellular response mounted by the action of lipopolysaccharide endotoxins mediated by TLR-4. As disclosed in the specification and as known to the researcher in the field, one may measure such responses through a variety of means, each identifying and measuring responses at a particular point in the signaling pathway.

7. The examiner has rejected several claims because the examiner believes that the name "TLR-4" is not definitive of particular proteins. The examiner states that insufficient structural

and functional properties have been presented in the specification to allow the proper identification of a TLR-4 protein. I do not find this to be the case.

8. Contrary to the examiner's position, my reading of the application provides me with at least sufficient structural and functional properties by which to identify a protein as TLR-4 or its homolog. The particular name associated with TLR-4 and its homologs is not determinative of their identity. Rather, its is their structure, primarily the similarity of the amino acid sequences among members of the TLR-4 family, and their function, primarily their role in mediating responses to endotoxins, that identifies TLR-4 polypeptides.

9. First, the family of TLR-4 receptors share high sequence similarities in specific domains, identifiable by their shared sequence motifs, as provided by the application. See, for example, pages 110-122.

10. Second, the domains of TLR-4 have specific functions, as described in the application. Primarily, TLR-4 polypeptides act to signal the presence of LPS. TLR-4 is an essential component of the signaling process and its ability to so signal is one of its defining functions.

11. Lastly, researchers in the field of LPS signaling are well aware of the remaining members of the toll-like receptor family, generally, and are able to identify TLR-4 and its homologs using the structural and functional features shared by all TLR-4 polypeptides.

12. The examiner has rejected the claims on the grounds that practice of the invention as claimed would require undue experimentation. Particularly, the examiner asserts that the specification does not provide for methods of measuring LPS mediated responses other than through measuring altered expression of TLR-4 and therefore does not provide methods for

identification of compounds that may modulate LPS responses by any other mechanism than altering TLR-4 expression. I do not find this to be the case.

13. Contrary to the examiner's position, it is well within the skill of one in the field of endotoxin and cellular biology to screen for compounds that modulate the LPS responses through their action upon TLR-4 beyond up or down regulation of TLR-4 expression. The screening of candidate compounds for their effects upon protein action and interaction is routine in the field. In view of the contents of the application, such screening is not limited to those compounds that may alter TLR-4 expression. Indeed, the general expectation of researchers performing such screens is that they will produce small compounds that specifically alter the binding specificity, signaling capacity, or other functional property of the target protein, in this case, TLR-4.

14. The specification clearly sets forth assays of TLR-4 activity in the LPS response pathway that can be used by one of ordinary skill in the art to determine, without undue experimentation, whether or not such candidate compounds modulate the action of TLR-4 independently of any action upon TLR-4 expression. For example, such assays are described in the specification at pages 87-88. Furthermore, these and further assays are available through the general knowledge of one of skill in the field of endotoxin biology.

15. I expect, based upon my skill and training in the areas of endotoxin and cellular biology that an ordinary researcher in these areas would be able to routinely practice the claimed invention following the guidance provided in the application and using the knowledge generally available in endotoxin biology.

16. I declare that all statements made of my knowledge are true and all statements made on the information are believed to be true; and, further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Date: 9/26/02

David D.Chaplin, M.D., Ph.D.

CURRICULUM VITAE

Name:	David Dunbar Chaplin
Date of Birth:	August 28, 1952
Place of Birth:	London, England
Citizenship:	U.S.A.
Social Security Number:	067-38-2393
Home Address:	406 Wildwood Lane Indian Springs, AL 35124
Current Position:	Charles H. McCauley Professor and Chair Department of Microbiology University of Alabama at Birmingham 845 19th Street South, BBRB 276/11 Birmingham, AL 35294-2170
Undergraduate Education:	Harvard College Cambridge, Massachusetts A.B. June, 1973
Graduate Education:	Washington University St. Louis, Missouri M.D. May, 1980 Ph.D. May, 1980

Post-doctoral Training:

1982-1984	Harvard Medical School, Department of Genetics Boston, Massachusetts, Fellow
1980-1982	University of Texas, Southwestern Medical School Parkland Memorial Hospital, Dallas, Texas Internal Medicine Residency

Academic Appointments:

2001-present	Chairman, University of Alabama at Birmingham, Department of Microbiology, Birmingham, AL
2001-present	Senior Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham
1995-2001	Associate Physician, Barnes-Jewish Hospital, University of Washington, St. Louis, MO
1995-2001	Professor, Washington University School of Medicine, Departments of Medicine, Genetics, and Molecular Microbiology, St. Louis, MO
1994-2001	Chief, Div. of Allergy and Immunology, Washington University School of Medicine, Department of Medicine

Academic Appointments (continued):

1992-1995	Assoc. Professor, Washington University School of Medicine, Department of Genetics, St. Louis, MO
1991-1995	Assoc. Professor, Washington University School of Medicine, Department of Medicine and Molecular Microbiology, St. Louis, MO
1989-1992	Asst. Professor, Washington University School of Medicine, Department of Genetics, St. Louis, MO
1984-1995	Assistant Physician, Barnes-Jewish Hospital, University of Washington, St. Louis, MO
1984-2001	Assoc. Investigator, Howard Hughes Medical Institute
1984-1991	Asst. Professor, Washington University School of Medicine, Dept. of Medicine and Molecular Microbiology, St. Louis, MO

Honors/Awards:

2001	Fellow, American Academy of Allergy, Asthma and Immunology
1997	Association of American Physicians
1995-1998	Councilor, American Society for Clinical Investigation
1993	Fellow, American Association for the Advancement of Science
1993	American Society for Clinical Investigation
1982-1984	Jane Coffin Childs Memorial Fund for Medical Research Fellowship
1980	Alpha Omega Alpha
1974-1980	Medical Scientist Trainee

Scientific Organizations:

2001-present	Secretary, American Academy of Allergy, Asthma and Immunology, Basic and Clinical Immunology Interest Section,
1994-2001	Associate Editor, Journal of Immunology
1993-present	International Cytokine Society
1991-present	American Academy of Allergy, Asthma and Immunology
1991-1996	Associate Editor, Diabetes
1989-1991	Associate Editor, The New Biologist
1989-present	American Society of Human Genetics
1986-present	American Association of Immunologists
1985-present	American Federation of Clinical Research
1984-present	American Association for the Advancement of Science

Keywords: Inflammatory Cytokines; TNF; IL-1; Asthma Pathogenesis; Lymphoid Tissue Development; Th Cell Function; Germinal Centers; Follicular Dendritic Cells

Publications:

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115. Verbsky, J.W., Randolph, D.A., Shornick, L.P., and Chaplin, D.D. (2002): Nonhematopoietic expression of Janus Kinase 3 is required for efficient recruitment of Th2 lymphocytes and eosinophils in OVA-induced airway inflammation. *J Immunol* 168:2475-2482.

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Invited Lectures:

Jan. 26, 1984	The Royal Society of London, Biochemistry and Genetics of Complement: Cloning and expression of murine C4 and Slp.
Dec. 12, 1988	Univ. of Missouri, Dept. of Microbiology: Molecular immunology of Interleukin-1.
Dec. 17, 1991	Univ. of Texas Medical Branch at Galveston: Interleukin-1, a secreted cytokine?
Nov. 7, 1994	National Workshop on Alopecia Areata: HLA-linked skin disease: classical HLA genes or novel genes within HLA?
Jan. 31, 1995	Ohio State Univ.: Molecular Analysis of the HLA Complex.
Aug. 25, 1995	BASF BioResearch Corp: Gene Targeting to Define the Role of IL-1β *in vivo.*
Feb. 15, 1996	Barnes-Jewish Medical Grand Rounds: Gene Targeting to Define the *in Vivo* Functions of Cytokines
May 10, 1996	6th International Congress, TNF and Related Molecules, Rhodes, Greece: Lymphotoxin-α-Deficient and TNF-Receptor I-Deficient Mice Define Developmental and Functional Characteristics of Germinal Centers.
May 21, 1996	St. Louis Jewish Hospital Grand Rounds: Gene Targeting to Define the *in Vivo* Functions of Cytokines
Oct. 28, 1996	Chairman, Inflammation Research Association Conference Session: Targets and Cytokine Action
Dec. 16, 1996	University of Washington Immunology Program: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Dec. 17, 1996	Immunex Corp.: Essential Role of IL-1β in Contact Hypersensitivity Responses
Feb. 13, 1997	Biogen Corp.: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Mar. 20, 1997	New York University School of Medicine/Skirball Institute: Essential Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Apr. 11, 1997	University of Utah, Developmental Biology Program: Cytokine Signals for Lymphoid Tissue Development
May 21, 1997	Pfizer Corp.: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
May 22, 1997	Inflammation Research Association: Induction of IL-1 During Apoptosis

June 24, 1997	FASEB Conference on Autoimmunity: Cytokine Signals for Lymphoid issue Development
July 1, 1997	Gordon Conference: Lymphotoxin, a Primary Determinant of Lymphoid Tissue Structure
Oct.8, 1997	National Jewish Center for Immunology and Respiratory Diseases: Lymphotoxin, a Primary Determinant of Lymphoid Tissue Structure
Dec. 3, 1997	Duke University, Department of Immunology: Role of Lymphotoxin in Peripheral Lymphoid Tissue Development
Jan. 27, 1998	37th Midwinter Immunology Conference, Asilomar: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Feb. 19, 1998	University of North Carolina, Department of Microbiology: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Mar. 2, 1998	University of Rochester, Department of Pediatrics: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
May 20, 1998	7th International TNF Congress, Hyannis: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
June 23, 1998	FASEB Conference on Lymphocytes and Antibodies: TNF/LT Family Members as Signals for Lymphoid Tissue Development
June 26, 1998	International Union of Immunological Societies, Symposium on Primary Immunodeficiency Diseases: Cytokine Signals for the Development of Primary B Cell Follicle Structure
Sept. 9, 1998	St. Jude Children's Research Hospital, Department of Immunology: Lymphotoxin-Dependent Signals Controlling Peripheral Lymphoid Tissue Development
Oct. 27, 1998	International Cytokine Society, Jerusalem: Lymphotoxin-Dependent Signals Regulating Primary B Cell Follicle Structure and Function
Dec. 7, 1998	Washington University Center for Immunology Seminar: Signals Controlling Normal Lymphoid Tissue Structure and Function
Dec. 9, 1998	Wistar Institute: Lymphotoxin, a Major Determinant for Normal Secondary Lymphoid Tissue Development and Function
Jan. 26, 1999	Vanderbilt University, Department of Microbiology and Immunology: Signals Controlling Normal Lymphoid Tissue Structure and Function

Feb. 11, 1999	Keystone Conference: B Lymphocyte Biology and Disease TNF Family Members in Formation of Primary Lymphoid Follicles
Feb. 27, 1999	American Academy of Allergy, Asthma and Immunology, 55th Annual Meeting: Synergy of Th1 and Th2 Cells in Experimental Eosinophilic Airway Inflammation
Mar. 15, 1999	University of Toronto, Immunology Department Seminar Series: Cellular and Molecular Determinants of Peripheral Lymphoid Tissue Structure and Function
May 8, 1999	Nikolas Symposium, Athens, Greece: Cytokines and Lymphoid Tissue Development
Sept. 25, 1999	National Residency Education Program, American Association of Allergy, Asthma, and Immunology, St. Louis, MO: Allergy-Immunology: from Bench to Bedside.
Oct. 22, 1999	Allergy Abroad, Paris, France: Cooperation Between T Helper Cells in Allergic Airway Inflammation
Oct. 26, 1999	Allergy Abroad, Lyon, France: Control of Lymphocyte Movement and Function by Chemokines
Oct. 29, 1999	Allergy Abroad, Montpellier, France Organization and Function of Secondary Lymphoid Tissues
Nov. 9, 1999	Stanford University, Program in Immunology Seminar: Regulation of Lymphoid Tissue Structure and Function
Nov. 30, 1999	Kyoto University, Department of Molecular Genetics: Regulation of Lymphoid Tissue Structure and Function
Dec. 2, 1999	Kyoto, Japan, 29th Annual Meeting of the Japanese Society for Immunology, Symposium on Lymphocyte Development in Germinal Centers: Targeting within Secondary Lymphoid Tissues and Control of Antibody Responses
Apr. 5, 2000	University of Alabama at Birmingham, Department of Microbiology Regulation of Lymphoid Tissue Structure and Function
Apr. 17, 2000	NIAID/NCI Symposium: Cells of the Marginal Zone – Origins, Function and Neoplasia, Bethesda, MD: Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
May 13, 2000	AAI Annual Meeting, Seattle, WA. Major Symposium Co-Chair: Molecular Mechanisms of Lymphoid Organogenesis. Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
Aug. 19, 2000	Clinical Allergy for the Practicing Physician, St. Louis, MO. DNA Vaccines

Sept. 9, 2000	1st International Workshop on Nucleotides and Their Receptors in the Immune System, Ferrara, Italy Is apoptosis required for IL-1 action *in vivo*?
Oct. 3, 2000	Howard Hughes Medical Institute: Infection and Immunity Molecular Determinants of Spleen Follicle Structure and Function
Oct. 25, 2000	University of Iowa, Department of Microbiology Regulation of secondary lymphoid tissue follicle structure and function by lymphotoxin
Jan. 17, 2001	Albert Einstein College of Medicine, Division of Biological Sciences Seminar Series Molecular Determinants of Spleen Follicle Structure and Function
Mar. 12, 2001	Washington University Center for Immunology Seminar: Regulation of Secondary Lymphoid Tissue Structure and Function by Lymphotoxin and TNF
Mar. 18, 2001	57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology, New Orleans, LA: Grand Seminar. Regulation of Secondary Lymphoid Tissue Structure and Function by Lymphotoxin
Apr. 19, 2001	New York University Immunology Program Seminar: Mechanisms Regulating Th2-dependent Inflammation in Peripheral Tissues
May 23, 2001	Mucosal Immunology at the 21st Century, Perdido Beach, AL: Plasticity of Secondary Lymphoid Tissue Structures
June 7, 2001	NIH/NIAID Asthma Center Directors Meeting, Bethesda, MD: Regulation of T Helper Cell Recruitment to Peripheral Tissues
July 23, 2001	11th International Congress of Immunology, Stockholm, Sweden: Symposium on Antigen Processing and Presentation at Mucosal Surfaces. Control of Lymphoid Tissue Structure and Function by LT and TNF
Nov. 6, 2001	EU and NIH Conference, Siena, Italy: Potential Impact of New Technologies on Vaccination in Early Life. Signals for Development of Secondary Lymphoid Organs
Dec. 5, 2001	British Society for Immunology Annual Congress, Harrogate, UK: Plenary Speaker. Recruitment of Th2 Cells to Peripheral Sites *in vivo*
Jan. 22, 2002	Department of Microbiology, University of Alabama at Birmingham: Recruitment of Th2 Cells to Peripheral Sites *in vivo*
Feb. 8, 2002	9th International Conference on Lymphocyte Traffic and Homeostasis, Newport Beach, CA: Structural Elements Regulating Lymphocyte Trafficking to and in the Spleen
Mar. 2, 2002	58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology,

New York, NY: Role of Inflammation in Recruitment of Th2 Lymphocytes to the Lung

June 9, 2002 FASEB Conference, Anatomy of the Immune Response *in vivo*, Snowmass, CO: Lymphocyte Trafficking Patterns in the Spleen